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Invention: GENETIC POLYMORPHISMS IN THE HUMAN NEUROKININ 1 RECEPTOR GENE AND THEIR USE IN DIAGNOSIS AND TREATMENT OF DISEASES

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SPECIFICATION

GENETIC POLYMORPHISMS IN THE HUMAN NEUROKININ 1 RECEPTOR GENE AND THEIR USES IN DIAGNOSIS AND TREATMENT OF DISEASES

This invention relates to polymorphisms in the human NK1R gene and corresponding novel allelic polypeptides encoded thereby. The invention also relates to methods and materials for analysing allelic variation in the NK1R gene and to the use of said polymorphism in the diagnosis and treatment of NK1R ligand mediated diseases, such as asthma.

The reader is referred to the following publications for background information: Primary structure and gene organization of human substance P and neuromedin K receptors, K Takahashi *et al* Eur J Biochem **204**, 1025-1033 (1992); Differential activation of intracellular effector by two isoforms of the human neurokinin-1 receptor, TM Fong *et al* Molecular Pharmacology **41**,24-30 (1992); Human Substance P receptor (NK-1): organisation of the gene, chromosome localization, and functional expression of cDNA clones, Gerard *et al* Biochemistry **30**, 10640-10646 (1991); Isolation and characterization of the human lung NK-1 receptor cDNA, Hopkins *et al*, Biochem Biophys Res Commun **180**,110-1117 (1991); Mutational analysis of neurokinin receptor function. TM Fong *et al* Can J Physiol Pharmacol **73**, 860-865 (1995); Structure and function of G protein-coupled receptors, CD Strader *et al* Annual Reviews Biochemistry **63**,101-132 (1994); The evolution and structure of aminergic G protein-coupled receptors, D Donnelly *et al* Receptors and Channels **2**, 61-78 (1994).

NK1R polypeptide is known to exist in 2 isoforms, which are possibly alternatively spliced variants of a single NK1R gene, see TM Fong *et al* Molecular Pharmacology **41**,24-30 (1992). A cDNA encoding NK1R has been published in International patent application WO 92/16547, Children's Medical Center; and in European patent application EP 510 878, Merck.

The complete genomic sequence of NK1R is not presently known but regions thereof containing exons 1, 3 & 5 have been published by EMBL as follows: Exon 1, Accession Number X 65177, 2472 bp; Exon 3, Accession Number X65179, 373 bp; Exon 5, Accession Number X65181, 3929 bp. Apart from Accession Number X 65177, all positions herein relate to the positions indicated therein unless stated otherwise or apparent from the context. The inventors have discovered that part of the sequence presented in EMBL Accession Number X65177 is incorrect. Sequencing of genomic PCR products by the present inventors, has shown that the nucleotide sequence from positions 262 - 758 of EMBL Accession Number X65177 is incorrect and a Blast search of the data bases has shown that this

erroneous sequence actually corresponds to positions 1231-1729 of EMBL Accession Number U37688 which encodes a gene similar to the human c-myc proto-oncogene. None of the specific polymorphisms identified herein however, fall within this erroneous sequence.

A sequence containing the promoter region of the human NK1R gene has been published in "Structure, expression and second messenger- mediated regulation of the human and rat substance P receptors and their genes" JE Krause et al Regulatory Peptides 46 , 59-66 (1993), (see Figure 2 - Conservation of the human and rat Substance P receptor gene putative promoter regions). The present inventors have confirmed that this sequence is correct. No accession number has been assigned to this published sequence. In view of the sequence error in EMBL X 65177, the corrected sequence is included herein as SEQ ID No. 1. This sequence has been used as the reference sequence for locating the position of the novel promoter and exon 1 (+ intron junction region) polymorphic variants of the NK1R gene identified herein.

With respect to SEQ ID No.1, nucleotides 1 - 261 correspond to sequences 1 - 261 in X 65177, sequence 262-833 replaces sequence 262-758 of X 65177 (resulting in an addition of 75 nucleotides to the complete sequence length) and sequence 834 - 2547 corresponds to sequence 759- 2472 of X 65177. Exon 1 ATG starts at position 2029 and ends at position 2417.

One approach is to use knowledge of polymorphisms to help identify patients most suited to therapy with particular pharmaceutical agents (this is often termed "pharmacogenetics") . Pharmacogenetics can also be used in pharmaceutical research to assist the drug selection process. Polymorphisms are used in mapping the human genome and to elucidate the genetic component of diseases. The reader is directed to the following references for background details on pharmacogenetics and other uses of polymorphism detection: Linder *et al.* (1997), Clinical Chemistry, **43**, 254; Marshall (1997), Nature Biotechnology, **15**, 1249; International Patent Application WO 97/40462, Spectra Biomedical; and Schafer *et al.* (1998), Nature Biotechnology, **16**, 33.

A haplotype is a set of alleles found at linked polymorphic sites (such as within a gene) on a single (paternal or maternal) chromosome. If recombination within the gene is random, there may be as many as 2^n haplotypes, where 2 is the number of alleles at each SNP and n is the number of SNPs. One approach to identifying mutations or polymorphisms which are correlated with clinical response is to carry out an association study using all the haplotypes

that can be identified in the population of interest. The frequency of each haplotype is limited by the frequency of its rarest allele, so that SNPs with low frequency alleles are particularly useful as markers of low frequency haplotypes. As particular mutations or polymorphisms associated with certain clinical features, such as adverse or abnormal events, are likely to be of low frequency within the population, low frequency SNPs may be particularly useful in identifying these mutations (for examples see: Linkage disequilibrium at the cystathionine beta synthase (CBS) locus and the association between genetic variation at the CBS locus and plasma levels of homocysteine. *Ann Hum Genet* (1998) **62**:481-90, De Stefano V, Dekou V, Nicaud V, Chasse JF, London J, Stansbie D, Humphries SE, and Gudnason V; and Variation at the von willebrand factor (vWF) gene locus is associated with plasma vWF:Ag levels: identification of three novel single nucleotide polymorphisms in the vWF gene promoter. *Blood* (1999) **93**:4277-83, Keightley AM, Lam YM, Brady JN, Cameron CL, Lillicrap D).

Clinical trials have shown that patient response to treatment with pharmaceuticals is often heterogeneous. Thus there is a need for improved approaches to pharmaceutical agent design and therapy.

Point mutations in polypeptides will be referred to as follows: natural amino acid (using 1 or 3 letter nomenclature) , position, new amino acid. For (a hypothetical) example, "D25K" or "Asp25Lys" means that at position 25 an aspartic acid (D) has been changed to lysine (K). Multiple mutations in one polypeptide will be shown between square brackets with individual mutations separated by commas.

The present invention is based on the discovery of single nucleotide polymorphisms (SNPs) in the NK1R gene. As defined herein, the NK1R gene includes exon coding sequence, intron sequences intervening the exon sequences and, 3' and 5' untranslated region (3' UTR and 5' UTR) sequences, including the promoter element of the NK1R gene.

According to one aspect of the present invention there is provided a method for the diagnosis of a single nucleotide polymorphism in NK1R in a human, which method comprises determining the sequence of the nucleic acid of the human at one or more of positions:

2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

and determining the status of the human by reference to polymorphism in the NK1R gene.

According to another aspect of the present invention there is provided a method for diagnosis of a single nucleotide polymorphism in NK1R in a human, which method comprises determining the sequence of the nucleic acid of the human at one or more positions:

2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

461 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

495 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

600 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

809 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

and determining the status of the human by reference to polymorphism in NK1R or its 3′ untranslated region.

According to another aspect of the present invention there is provided a method for diagnosis of one or more single nucleotide polymorphism(s) in NK1R gene in a human, which method comprises determining the sequence of the nucleic acid of the human at one or more positions:

2361 in exon 1 as defined by the position in SEQ ID No. 1;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

461 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

495 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

600 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

809 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

1371 in the promoter element as defined by the position in SEQ ID No. 1;

and determining the status of the human by reference to polymorphism in NK1R.

The term human includes both a human having or suspected of having a NK1R ligand mediated disease and an asymptomatic human who may be tested for predisposition or susceptibility to such disease. At each position the human may be homozygous for an allele or the human may be a heterozygote.

In one embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 2361 in exon 1 is presence of C and/or T.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 271 near exon 3 is presence of G and/or T.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 272 near exon 3 is presence of A and/or a single base deletion at this position.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 245 in exon 5 is presence of C and/or a single base deletion at this position. This results in premature termination and loss of C-terminal 26 amino acids (see Example 1 below). Testing for the presence of this polymorphism is especially preferred because, without wishing to be bound by theoretical considerations, of its association with a significant loss of amino acids.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 461 in the 3'UTR is presence of G and/or C.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 495 in the 3'UTR is the presence of T and/or a single base insertion of A at this position.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 600 in the 3'UTR is presence of A and/or G.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 809 in the 3'UTR is presence of C and/or T.

In another embodiment of the invention preferably the method for diagnosis described herein is one in which the single nucleotide polymorphism at position 1371 in the promoter element is presence of A and/or G.

When considering positional relationships in the presence of a single base deletion or insertion the introduction of an appropriate gap is required in accordance with established techniques.

In another aspect of the invention we provide a method for the diagnosis of NK1R ligand-mediated disease, which method comprises:

i) obtaining sample nucleic acid from an individual,

detecting the presence or absence of a variant nucleotide at one or more of positions:

2361 in exon 1 as defined by the position in SEQ ID No. 1;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

461 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

495 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

600 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

809 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

1371 in the promoter element as defined by the position in SEQ ID No. 1;

and determining the status of the human by reference to polymorphism in NK1R.

The method for diagnosis is preferably one in which the sequence is determined by a method selected from allele specific amplification (i.e. ARMS™-allele specific amplification; ARMS referring to amplification refractory mutation system), allele specific hybridisation (ASH), oligonucleotide ligation assay (OLA) and restriction fragment length polymorphism (RFLP).

The status of the human may be determined by reference to allelic variation at any one, two, three, four, five, six, seven, eight or all nine positions. The status of the human may also be determined by one or more of the specific polymorphisms identified herein in combination with one or more other SNP's.

NK1 antagonists have been explored by Glaxo, Pfizer, Merck, Parke-Davis, Lilly, RPR, and Sanofi, primarily for CNS indications. In a recent clinical trial it was reported that a single dose of Pfizer CP-122,721 inhibits emesis associated with chemotherapy and was well tolerated with no adverse effects (Kris *et al*, JNCI, **89**, 817, 1997). Most recently Merck has announced positive Phase II studies with a NK1 antagonist in depression/anxiety.

It is believed that a dual NK1/ NK2 receptor antagonist will have some clinical utility, particularly for asthma. As compared with conventional therapies, it is expected that a dual NK1/NK2 receptor antagonist will better control airways hyper-responsiveness and neurogenic inflammation (extravasation and hypersecretion), both of which are characteristic manifestations of asthma. This multifaceted approach improves upon other therapies that are designed to treat only a single clinical manifestation of this disease. Other therapeutic opportunities for NK1/NK2 antagonist exist in pain, migraine, anxiety, depression, urinary incontinence, and inflammatory bowel disease.

Four companies have published on mixed NK1/NK2 receptor antagonists. Two of the compounds are peptides: FK-224 (Fujisawa) and S16474 (Servier). The other two, MDL-105,212 (Marion Merrell Dow) and a recent compound from Merck, are structurally related to the selective NK2 antagonist, SR48968 (Sanofi). Neurokinin receptor antagonists have been reviewed by C J Swain (1996) in Exp. Opin. Ther. Patents, **6**, 367-378; and by Elliot & Seward (1997) in Exp. Opin. Ther. Patents, **7**, 43-54.

The test sample of nucleic acid is conveniently a sample of blood, bronchoalveolar lavage fluid, sputum, urine or other body fluid or tissue obtained from an individual. It will be appreciated that the test sample may equally be a nucleic acid sequence corresponding to the sequence in the test sample, that is to say that all or a part of the region in the sample nucleic acid may firstly be amplified using any convenient technique e.g. PCR, before analysis of allelic variation.

It will be apparent to the person skilled in the art that there are a large number of analytical procedures which may be used to detect the presence or absence of variant nucleotides at one or more polymorphic positions of the invention. In general, the detection of allelic variation requires a mutation discrimination technique, optionally an amplification reaction and optionally a signal generation system. Table 1 lists a number of mutation detection techniques, some based on the polymerase chain reaction (PCR). These may be used in combination with a number of signal generation systems, a selection of which is listed in Table 2. Further amplification techniques are listed in Table 3. Many current methods for the detection of allelic variation are reviewed by Nollau *et al.*, Clin. Chem. **43**, 1114-1120, 1997; and in standard textbooks, for example "Laboratory Protocols for Mutation Detection", Ed. by U. Landegren, Oxford University Press, 1996 and "PCR", 2nd Edition by Newton & Graham, BIOS Scientific Publishers Limited, 1997.

Abbreviations:

ALEX™	Amplification refractory mutation system linear extension
APEX	Arrayed primer extension
ARMS™	Amplification refractory mutation system
b-DNA	Branched DNA
CMC	Chemical mismatch cleavage
bp	base pair
COPS	Competitive oligonucleotide priming system
DGGE	Denaturing gradient gel electrophoresis
FRET	Fluorescence resonance energy transfer
LCR	Ligase chain reaction
MASDA	Multiple allele specific diagnostic assay
MCP-1	Monocyte chemoattractant protein 1
NASBA	Nucleic acid sequence based amplification
NK	Neurokinin
NK1R	Neurokinin 1 receptor
NK2R	Neurokinin 2 receptor
OLA	Oligonucleotide ligation assay
PCR	Polymerase chain reaction
PTT	Protein truncation test
RFLP	Restriction fragment length polymorphism
SDA	Strand displacement amplification
SERRS	Surface enhanced raman resonance spectroscopy
SNP	Single nucleotide polymorphism
SSCP	Single-strand conformation polymorphism analysis
SSR	Self sustained replication
TGGE	Temperature gradient gel electrophoresis
3′ UTR	3′ untranslated region

Table 1 - Mutation Detection Techniques

General: DNA sequencing, Sequencing by hybridisation

Scanning: PTT*, SSCP, DGGE, TGGE, Cleavase, Heteroduplex analysis, CMC, Enzymatic mismatch cleavage

* Note: not useful for detection of promoter polymorphisms.

Hybridisation Based

Solid phase hybridisation: Dot blots, MASDA, Reverse dot blots, Oligonucleotide arrays (DNA Chips)

Solution phase hybridisation: Taqman™ - US-5210015 & US-5487972 (Hoffmann-La Roche), Molecular Beacons - Tyagi *et al* (1996), Nature Biotechnology, **14**, 303; WO 95/13399 (Public Health Inst., New York)

Extension Based: ARMS™-allele specific amplification (as described in European patent No. EP-B-332435 and US patent No. 5,595,890), ALEX™ - European Patent No. EP 332435 B1 (Zeneca Limited), COPS - Gibbs *et al* (1989), Nucleic Acids Research, **17**, 2347.

Incorporation Based: Mini-sequencing, APEX

Restriction Enzyme Based: RFLP, Restriction site generating PCR

Ligation Based: OLA

Other: Invader assay

Table 2 - Signal Generation or Detection Systems

Fluorescence: FRET, Fluorescence quenching, Fluorescence polarisation - United Kingdom Patent No. 2228998 (Zeneca Limited)

Other: Chemiluminescence, Electrochemiluminescence, Raman, Radioactivity, Colorimetric, Hybridisation protection assay, Mass spectrometry, SERRS - WO 97/05280 (University of Strathclyde).

Table 3 - Further Amplification Methods

SSR, NASBA, LCR, SDA, b-DNA

Preferred mutation detection techniques include ARMS™-allele specific amplification, ALEX™, COPS, Taqman, Molecular Beacons, RFLP, OLA, restriction site based PCR and FRET techniques.

Particularly preferred methods include ARMS™-allele specific amplification, OLA and RFLP based methods. ARMS™-allele specific amplification is an especially preferred method.

ARMS™-allele specific amplification (described in European patent No. EP-B-332435, US patent No. 5,595,890 and Newton et al. (Nucleic Acids Research, Vol. 17, p.2503; 1989)), relies on the complementarity of the 3' terminal nucleotide of the primer and its template. The 3' terminal nucleotide of the primer being either complementary or non-complementary to the specific mutation, allele or polymorphism to be detected. There is a selective advantage for primer extension from the primer whose 3' terminal nucleotide complements the base mutation, allele or polymorphism. Those primers which have a 3' terminal mismatch with the template sequence severely inhibit or prevent enzymatic primer extension. Polymerase chain reaction or unidirectional primer extension reactions therefore result in product amplification when the 3' terminal nucleotide of the primer complements that of the template, but not, or at least not efficiently, when the 3' terminal nucleotide does not complement that of the template.

By way of example, a suitable allele specific primer (ARMS primer) capable of detecting/diagnosing the 2361 "T" polymorphism in Exon 1 is: 5'-GCAAGTTCCACAACTTCTTT-3' (SEQ ID No. 2). The 3' terminal nucleotide complementing the "A" polymorphism on the anti-sense template strand facilitates efficient primer extension with the suitable enzyme (preferably one lacking 3'-5' exonuclease activity).

In a further aspect, the diagnostic methods of the invention are used to assess the efficacy of therapeutic compounds in the treatment of NK1R ligand mediated diseases, such as asthma.

The polymorphisms identified in the present invention that occur in intron regions or in the promoter region are not expected to alter the amino acid sequence of the NK1 receptor, but may affect the transcription and/or message stability of the sequences and thus affect the level of the receptors in cells.

Assays, for example reporter-based assays, may be devised to detect whether one or more of the above polymorphisms affect transcription levels and/or message stability.

Individuals who carry particular allelic variants of the NK1R gene may therefore exhibit differences in their ability to regulate protein biosynthesis under different physiological conditions and will display altered abilities to react to different diseases. In addition, differences in protein regulation arising as a result of allelic variation may have a direct effect

on the response of an individual to drug therapy. The diagnostic methods of the invention may be useful both to predict the clinical response to such agents and to determine therapeutic dose.

In a further aspect, the diagnostic methods of the invention, are used to assess the predisposition and/or susceptibility of an individual to diseases mediated by NK1R ligands. The present invention may be used to recognise individuals who are particularly at risk from developing these conditions.

In a further aspect, the diagnostic methods of the invention are used in the development of new drug therapies which selectively target one or more allelic variants of the NK1R gene. Identification of a link between a particular allelic variant and predisposition to disease development or response to drug therapy may have a significant impact on the design of new drugs. Drugs may be designed to regulate the biological activity of variants implicated in the disease process whilst minimising effects on other variants.

In a further diagnostic aspect of the invention the presence or absence of variant nucleotides is detected by reference to the loss or gain of, optionally engineered, sites recognised by restriction enzymes. For example the polymorphism at position 271 and 272 in exon 3 can be detected by digestion with the restriction enzymes RsaI and Cac8I respectively. Engineered sites include those wherein the primer sequences employed to amplify the target sequence participates along with the nucleotide polymorphism to create a restriction site (see for example, Example 2 section 2 on 809 polymorphism in the 3' UTR (SEQ ID No. 6)).

According to another aspect of the present invention there is provided a nucleic acid comprising any one of the following polymorphisms:

the nucleic acid sequence of EMBL ACCESSION NO. X 65177 with T at position 2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;

the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with T at position 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with a single base deletion at position 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with a single base deletion at position 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one of the polymorphisms.

According to another aspect of the present invention there is provided a nucleic acid comprising any one of the following polymorphisms:

the nucleic acid sequence of EMBL ACCESSION NO. X 65177 with T at position 2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;

the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with T at position 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with a single base deletion at position 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with a single base deletion at position 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with C at position 461 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181;

the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with A inserted at position 495 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181;

the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with G at position 600 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181;

the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with T at position 809 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181;

or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one of the polymorphisms.

According to another aspect of the present invention there is provided a nucleic acid comprising any one of the following polymorphism containing sequences:

the nucleic acid sequence of SEQ ID No. 1 with T at position 2361 in exon 1 as defined by the position in SEQ ID No. 1; the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with T at position 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with a single base deletion at position 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with a single base deletion at position 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with C at position 461 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181;

the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with A inserted at position 495 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with G at position 600 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with T at position 809 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of SEQ ID No. 1 with G at position 1371 in the promoter element as defined by the position in SEQ ID No. 1; or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one of the polymorphisms.

Fragments are at least 17 bases, more preferably at least 20 bases, more preferably at least 30 bases.

The invention further provides nucleotide primers which can detect the polymorphisms of the invention.

According to another aspect of the present invention there is provided an allele specific primer capable of detecting a NK1R gene polymorphism of the invention.

An allele specific primer is used, generally together with a constant primer, in an amplification reaction such as a PCR reaction, which provides the discrimination between alleles through selective amplification of one allele at a particular sequence position e.g. as used for ARMS™ assays. The allele specific primer is preferably 17-50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

An allele specific primer preferably corresponds exactly with the allele to be detected but derivatives thereof are also contemplated wherein about 6-8 of the nucleotides at the 3' terminus correspond with the allele to be detected and wherein up to 10, such as up to 8, 6, 4, 2, or 1 of the remaining nucleotides may be varied without significantly affecting the properties of the primer. Often the nucleotide at the -2 and/or -3 position (relative to the 3' terminus) is mismatched in order to optimise differential primer binding and preferential extension from the correct allele discriminatory primer only.

Primers may be manufactured using any convenient method of synthesis. Examples of such methods may be found in standard textbooks, for example "Protocols for Oligonucleotides and Analogues; Synthesis and Properties," Methods in Molecular Biology Series; Volume 20; Ed. Sudhir Agrawal, Humana ISBN: 0-89603-247-7; 1993; 1st Edition. If required the primer(s) may be labelled to facilitate detection.

According to another aspect of the present invention there is provided an allele-specific oligonucleotide probe capable of detecting a NK1R gene polymorphism of the invention.

The allele-specific oligonucleotide probe is preferably 17- 50 nucleotides, more preferably about 17-35 nucleotides, more preferably about 17-30 nucleotides.

The design of such probes will be apparent to the molecular biologist of ordinary skill. Such probes are of any convenient length such as up to 50 bases, up to 40 bases, more conveniently up to 30 bases in length, such as for example 8-25 or 8-15 bases in length. In general such probes will comprise base sequences entirely complementary to the corresponding wild type or variant locus in the gene. However, if required one or more mismatches may be introduced, provided that the discriminatory power of the oligonucleotide probe is not unduly affected. The probes of the invention may carry one or more labels to facilitate detection, such as in Molecular Beacons.

According to another aspect of the present invention there is provided a diagnostic kit comprising one or more diagnostic probe(s) of the invention and/or diagnostic primer(s), particularly an allele-specific oligonucleotide primer, of the invention.

The diagnostic kits may comprise appropriate packaging and instructions for use in the methods of the invention. Such kits may further comprise appropriate buffer(s) and polymerase(s) such as thermostable polymerases, for example taq polymerase. Such kits may also comprise companion/constant primers and/or control primers or probes. A companion/constant primer is one that is part of the pair of primers used to perform PCR. Such primer usually complements the template strand precisely.

In another aspect of the invention, the single nucleotide polymorphisms of this invention may be used as genetic markers in linkage studies. This particularly applies to the polymorphism in exon 1 (position 2361 in SEQ ID No. 1) because of its relatively high frequency (see below). Further preferred polymorphisms of high frequency are at positions 461 and 809 in the 3'UTR (see example 2 below). Those polymorphisms that occur relatively infrequently are useful as markers of low frequency haplotypes.

According to another aspect of the present invention there is provided an allelic variant of human NK1R polypeptide having a C-terminal deletion of 26 amino acids.

According to another aspect of the present invention there is provided a method of treating a human in need of treatment with a NK1R ligand antagonist drug in which the method comprises:

i) diagnosis of a single nucleotide polymorphism in NK1R gene in the human, which diagnosis comprises determining the sequence of the nucleic acid at one or more of positions:
2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;
271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;
and determining the status of the human by reference to polymorphism in the NK1R gene; and

ii) administering an effective amount of a NK1R ligand antagonist.

According to another aspect of the present invention there is provided a method of treating a human in need of treatment with a NK1R ligand antagonist drug in which the method comprises:

(i) diagnosis of a single nucleotide polymorphism in the NK1R gene in the human, which diagnosis comprises determining the sequence of nucleic acid at one of more of positions:
2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;
271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;
245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;
461 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;
495 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;
600 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;
809 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;
and determining the status of the human by reference to polymorphism in the NK1R gene; and

(ii) administering an effective amount of a NK1R ligand antagonist.

According to another aspect of the present invention there is provided a method of treating a human in need of treatment with an NK1R ligand antagonist drug in which the method comprises:

(i) diagnosis of a single nucleotide polymorphism in the NK1R gene in the human, which diagnosis comprises determining the sequence of nucleic acid at one of more of positions:
2361 in exon 1 as defined by the position SEQ ID No. 1;
1371 in the promoter element as defined by the position in SEQ ID No. 1;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

461 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

495 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

600 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

809 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

and determining the status of the human by reference to polymorphism in the NK1R gene; and

(ii) administering an effective amount of a NK1R ligand antagonist.

Preferably determination of the status of the human is clinically useful. Examples of clinical usefulness include deciding which antagonist drug of drugs to administer and/or in deciding on the effective amount of the drug or drugs. The NK1R ligand antagonist may optionally also have activity at the NK2R.

According to another aspect of the present invention there is provided use of an NK1R ligand antagonist drug in preparation of a medicament for treating a NK1R ligand mediated disease in a human diagnosed as having a single nucleotide polymorphism at one or more of positions:

2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181.

According to another aspect of the present invention there is provided use of an NK1R ligand antagonist drug in preparation of a medicament for treating a NK1R ligand mediated disease in a human diagnosed as having a single nucleotide polymorphism at one or more of positions:

2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

461 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

495 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

600 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

809 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181.

According to another aspect of the present invention there is provided use of an NK1R ligand antagonist drug in preparation of a medicament for treating a NK1R ligand mediated disease in a human diagnosed as having a single nucleotide polymorphism at one or more of positions:

2361 in exon 1 as defined by the position in SEQ ID No. 1;

1371 in the promoter element as defined by the position in SEQ ID No. 1;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

461 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

495 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

600 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

809 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181.

According to another aspect of the present invention there is provided a pharmaceutical pack comprising an NK1R antagonist drug and instructions for administration of the drug to humans diagnostically tested for a single nucleotide polymorphism at one or more of positions:

2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181.

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2286 in exon 1 as defined by the position in EMBL ACCESSION NO. X 65177;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

461 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

495 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

600 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

809 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181.

According to another aspect of the present invention there is provided a pharmaceutical pack comprising an NK1R antagonist drug and instructions for administration of the drug to humans diagnostically tested for a single nucleotide polymorphism at one or more positions:

2361 in exon 1 as defined by the position in SEQ ID No. 1;

1371 in the promoter element as defined by the position in SEQ ID No. 1;

271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179;

245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181;

461 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

495 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

600 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181;

809 in the 3′ UTR as defined by the position in EMBL ACCESSION NO. X65181.

Testing for the presence of the polymorphism in exon 5 is especially preferred because, without wishing to be bound by theoretical considerations, of its resulting in a significant amino acid change in NK1R polypeptide (as explained herein).

The nucleic acid sequences of the invention, particularly those relating to and identifying the single nucleotide polymorphisms identified herein represent a valuable information source with which to identify further sequences of similar identity and characterise individuals in terms of, for example, their identity, haplotype and other sub-groupings, such as susceptibility to treatment with particular drugs. These approaches are most easily facilitated by storing the sequence information in a computer readable medium and then using the information in standard macromolecular structure programs or to search sequence databases using state of the art searching tools such as GCG (Genetics Computer Group), BlastX BlastP, BlastN, FASTA (refer to Altschul et al. J. Mol. Biol. **215**:403-410, 1990). Thus, the nucleic acid sequences of the invention are particularly useful as components in databases useful for sequence identity, genome mapping, pharmacogenetics and other search analyses. Generally, the sequence information relating to the nucleic acid sequences and polymorphisms of the invention may be reduced to, converted into or stored in a tangible medium, such as a computer disk, preferably in a computer readable form. For example, chromatographic scan data or peak data, photographic scan or peak data, mass spectrographic data, sequence gel (or other) data.

The invention provides a computer readable medium having stored thereon one or more nucleic acid sequences of the invention. For example, a computer readable medium is provided comprising and having stored thereon a member selected from the group consisting of: a nucleic acid comprising the sequence of a nucleic acid of the invention, a nucleic acid consisting of a nucleic acid of the invention, a nucleic acid which comprises part of a nucleic acid of the invention, which part includes at least one of the polymorphisms of the invention, a set of nucleic acid sequences wherein the set includes at least on nucleic acid sequence of the invention, a data set comprising or consisting of a nucleic acid sequence of the invention or a part thereof comprising at least one of the polymorphisms identified herein. The computer readable medium can be any composition of matter used to store information or data, including, for example, floppy disks, tapes, chips, compact disks, digital disks, video disks, punch cards and hard drives.

In a particular embodiment of the invention there is provided a computer readable medium having stored thereon a member selected from the group consisting of: a nucleic acid comprising SEQ ID No. 1; a set of nucleic acids wherein at least one of said sequences comprises SEQ ID No. 1; a data set representing a nucleic acid sequence comprising SEQ ID No. 1; a nucleic acid consisting of SEQ ID No. 1; a set of nucleic acids wherein at least one of said sequences consists of the sequence of SEQ ID No. 1; a nucleic acid comprising any part (i.e. fragment of at least 20 bases) of a sequence selected from the group consisting of: SEQ ID No. 1, EMBL ACCESSION NO. X 65177, EMBL ACCESSION NO. X 65179, EMBL ACCESSION NO. X 65179 or EMBL ACCESSION NO. X 65181, which part includes at least one of the polymorphisms identified herein.

A computer based method is also provided for performing sequence identification, said method comprising the steps of providing a nucleic acid sequence comprising a polymorphism of the invention in a computer readable medium; and comparing said polymorphism containing nucleic acid sequence to at least one other nucleic acid or polypeptide sequence to identify identity (homology), i.e. screen for the presence of a polymorphism. Such a method is particularly useful in pharmacogenetic studies and in genome mapping studies.

In a particular embodiment of the invention there is provided a method for performing sequence identification, said method comprising the steps of providing a nucleic acid sequence comprising a sequence selected from the group consisting of:

the nucleic acid sequence of SEQ ID No. 1 with T at position 2361 in exon 1 as defined by the position in SEQ ID No. 1; the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with T at position 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with a single base deletion at position 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with a single base deletion at position 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with C at position 461 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with A inserted at position 495 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with G at position 600 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with T at position 809 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of SEQ ID No. 1 with G at position 1371 in the promoter element as defined by the position in SEQ ID No. 1; or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one of the polymorphisms; and comparing said nucleic acid sequence to at least one other nucleic acid or polypeptide sequence to identify identity.

In another embodiment of the invention there is provided a method for performing sequence identification, said method comprising the steps of providing one or more of the following polymorphism containing nucleic acid sequences: the nucleic acid sequence of SEQ ID No. 1 with T at position 2361 in exon 1 as defined by the position in SEQ ID No. 1; the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with T at position 271 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65179 with a single base deletion at position 272 near exon 3 as defined by the position in EMBL ACCESSION NO. X 65179; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with a single base deletion at position 245 in exon 5 as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with C at position 461 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with A inserted at position 495 in the 3'UTR as defined by the

position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with G at position 600 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of EMBL ACCESSION NO. X 65181 with T at position 809 in the 3'UTR as defined by the position in EMBL ACCESSION NO. X 65181; the nucleic acid sequence of SEQ ID No. 1 with G at position 1371 in the promoter element as defined by the position in SEQ ID No. 1; or a complementary strand thereof or a fragment thereof of at least 20 bases comprising at least one of the polymorphisms, in a computer readable medium; and comparing said nucleic acid sequence to at least one other nucleic acid or polypeptide sequence to determine identity.

The invention will now be illustrated but not limited by reference to the following Examples. All temperatures are in degrees Celsius.

In the Examples below, unless otherwise stated, the following methodology and materials have been applied.

AMPLITAQ™, available from Perkin-Elmer Cetus, is used as the source of thermostable DNA polymerase.

General molecular biology procedures can be followed from any of the methods described in "Molecular Cloning - A Laboratory Manual" Second Edition, Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory, 1989).

Electropherograms were obtained in a standard manner: data was collected by ABI377 data collection software and the wave form generated by ABI Prism sequencing analysis (2.1.2).

EXAMPLES

Example 1

Identification of Polymorphisms

1. Methods

DNA Preparation

DNA was prepared from frozen blood samples collected in EDTA following protocol I (Molecular Cloning: A Laboratory Manual, p392, Sambrook, Fritsch and Maniatis, 2nd Edition, Cold Spring Harbor Press, 1989) with the following modifications. The thawed blood was diluted in an equal volume of standard saline citrate instead of phosphate buffered saline to remove lysed red blood cells. Samples were extracted with phenol, then

phenol/chloroform and then chloroform rather than with three phenol extractions. The DNA was dissolved in deionised water.

Template Preparation

Templates were prepared by PCR using the oligonucleotide primers and annealing temperatures set out below. The extension temperature was 72° and denaturation temperature 94°. Generally 50 ng of genomic DNA was used in each reaction and subjected to 35 cycles of PCR.

Exon 1 SEQ ID No. 1 2547 bp

	Fragment	Forward primer	Reverse Primer	Annealing Temp	Time
A).	2000-2467	2000-2019	2448-2467	58°	60s

	Fragment	Forward Primer	Reverse Primer	Annealing Temp	Time
B).	1168-1712	1168-1187	1693-1712	58°	60s

Exon 3 Accession Number X65179 373 bp

Fragment	Forward Primer	Reverse Primer	Annealing Temp	Time
14-318	14-33	299-318	58°	60s

Exon 5 Accession Number X65181 3929 bp

Fragment	Forward Primer	Reverse Primer	Annealing Temp	Time
18-417	18-38	398-417	58°	60s

For dye-primer sequencing these primers were modified to include M13 forward and reverse primer sequences (ABI protocol P/N 402114, Applied Biosystems) at the 5' end of the forward and reverse oligonucleotides respectively.

Dye Primer Sequencing

Dye-primer sequencing using M13 forward and reverse primers was as described in the ABI protocol P/N 402114 for the ABI Prism™ dye primer cycle sequencing core kit with

"AmpliTaq FS"™ DNA polymerase, modified in that the annealing temperature was 45° and DMSO was added to the cycle sequencing mix to a final concentration of 5 %.

The extension reactions for each base were pooled, ethanol/sodium acetate precipitated, washed and resuspended in formamide loading buffer.

4.25 % Acrylamide gels were run on an automated sequencer (ABI 377, Applied Biosystems).

2. Results

Exon 1 SEQ ID No. 1

A). Nucleotide 2361 C/T Phe (111) TTC/TTT

Allele frequency	TTC	47%
	TTT	53%

To precisely identify the location of the 2361 polymorphism, its relative location within SEQ ID No. 1 is as follows:

TGCAAGTTCCACAACTTCTTCCCCATCGCCGCTGTCTTCGC (SEQ ID No. 3)

2341 2361 2381

B). Nucleotide 1371 A/G

Allele frequency (37 individuals)	A	98.6%
	G	1.4 %

The polymorphism creates an recognition sequence (GGCCC) for the restriction enzyme, Sau 96I (New England Biolabs). A PCR product (position 1168-1712, 544 bp) containing the wild type sequence (AGCCC) will not be cleaved by Sau 96I (New England Biolabs). Digestion of a heterozygote product (A/GGCCC) will generate products of 203 bp, 341 bp and 540 bp. Digestion of a homozygous variant (GGCCC) will generate products of 203 bp and 341 bp.

Exon 3 Accession Number X 65179

Flanking intron

Nucleotide 271 G/T

Allele frequency	G	98.5%
	T	1.5%

Nucleotide 272 ΔA (note "Δ" indicates deletion)

Frequency	ΔA	4.5%

To precisely identify the location of the 271 and 272 polymorphisms, their relative locations within the X65179 wild-type sequence is as follows:

AAGTCTCTGCCAAGCGCAAGGTGAGCAGGGGACAGGCAGA (SEQ ID No. 4)
241 271 280

AAGTCTCTGCCAAGCGCAAGGTGAGCAGGGGACAGGCAGA (SEQ ID No. 5)
241 272 280

The G271T polymorphism creates a RsaI (GTAC) restriction enzyme recognition site. A PCR product (304 bp) containing the wild type sequence will not be digested by RasI. Digestion of a heterozyote product will give bands of 304bp, 257bp and 47 bp. Digestion of a homozygous variant product will generate bands of 257 bp, 47 bp.

The 272 ΔA polymorphism creates a Cac 8I (GCNNGC) restriction enzyme recognition site. A PCR product (304 bp) containing the wild type sequence will not be digested by Cac 8I. Digestion of a heterozyote product will give bands of 304bp, 258bp and 46 bp. Digestion of a homozygous variant product will generate bands of 258 bp, 46 bp.

Exon 5 Accession Number X 65181

Nucleotide 245 ΔC

Frequency ΔC 1.5%

This results in premature termination and loss of C-terminal 26 amino acids

...	379	380	381	382		379			
	S	L	D	L		S	W	T	stop
	TCC	CTG	GAC	CTG.....	→	... TCC	TGG	ACC	**TGA**

Unless otherwise indicated, all the allele frequencies were determined on the basis of analysis of 34 individuals.

Example 2

Identification of Polymorphisms

1. Methods

All PCR conditions and sequencing protocols are as described in Example 1. Allele frequencies were determined in a panel of 37 individuals.

Template Preparation

3′ UTR Accession Number X65181

Fragment	Forward Primer	Reverse Primer	Annealing Temp	Time
301-750	301-320	731-750	58°	60s
696-1144	696-715	1125-1144	58°	60s

2. Results

3′ UTR Accession Number: X65181

Nucleotide 461	G/C	Allele Frequency	G 72%
			C 28%

This polymorphism can be detected by digestion with restriction enzyme Dde I

GTTAG...	DdeI negative	....CTTAG...	Dde I positive
CAATC...		....GAATC...	

Nucleotide 495	A insertion	Allele Frequency	1.4%
Nucleotide 600	A/G	Allele Frequency	A 92%
			G 8%

This polymorphism can be detected by digestion with restriction enzyme Ban II

....AAGCCC..	Ban II negative	...GAGCCC...	Ban II positive
....TTCGGG..		...CTCGGG..	

Nucleotide 809	C/T	Allele Frequency	C 55%
			T 45%

This polymorphism can be detected by engineered restriction site Psp1406I (AACGTT) Engineered primer 787-808 **GGGTGAACAAAAGAAGGAACGT** (SEQ ID No. 6) co-operating with the polymorphism C/T to create the Psp1406I (AACGTT) site only if the "T" polymorphism is present in the target sequence.